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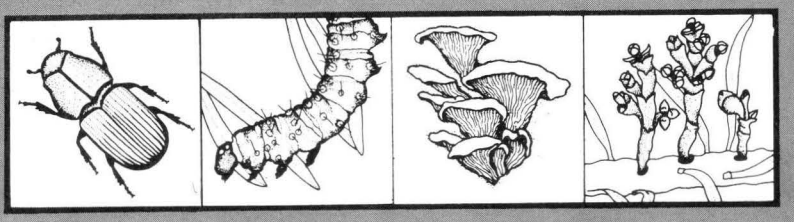

Report 90-4

3450
November 1989

OCCURRENCE, CHARACTERISTICS, AND DESCRIPTIONS OF *FUSARIUM* ISOLATES FROM DOUGLAS-FIR SEED AND SEEDLINGS

by

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ABSTRACT

Five different species of *Fusarium* were isolated from Douglas-fir seed and container-grown seedlings at two nurseries in northern Idaho. Nearly 90 percent of the isolates were *F. acuminatum* and *F. oxysporum*. Other isolated species included *F. avenaceum*, *F. sambucinum*, and *F. tricinctum*. Most *F. acuminatum* isolates were obtained from diseased and non-diseased seed and young diseased germinants, whereas most *F. oxysporum* isolates were found on roots of diseased and non-diseased seedlings either a few weeks to several months old. Temperature-growth relationships varied among the different *Fusarium* species tested. Most *F. oxysporum* isolates consistently grew faster at higher temperatures (34°C). Most *F. acuminatum* and *F. avenaceum* only grew at between 15-27°. All *F. sambucinum* isolates grew at from 5-34°C. Morphological characteristics of cultures and problems with taxonomic classification of isolates are discussed.

INTRODUCTION

Fusarium spp. cause important disease in forest tree nurseries. Early investigations in bareroot nurseries (Gifford 1911; Hartig 1892; Hartley 1921; Hartley and others 1918; Hartley and Pierce 1917; Rathbun 1918; Spaulding 1914) identified several *Fusarium* species as associates of damping-off and root diseases of seedlings. Disease etiology was established, and the most important pathogenic species was usually identified as *F. oxysporum* Schlect. (Bloomberg 1971; Bloomberg 1976; Hangyal 1971; Hocking 1968; Matuo and Chiba 1966; Tint 1945a; Vaartaja 1967; Vaartaja and Bumbieris 1967; Vaartaja and Cram 1956). Extensive epidemiological investigations of *F. oxysporum* in bareroot nurseries (Bloomberg 1973; Bloomberg 1985; Edmonds and Heather 1973; Lock 1973; Rathbun-Gravatt 1925; Shea and Rediske 1961; Tint 1945a, 1945c; Vaartaja 1952) provided important insight into behavior of pathogenic strains and conditions of host susceptibility leading to infection and disease expression. Further investigations provided valuable clues regarding approaches to disease management to reduce losses (Bloomberg 1981; Hartley and Pierce 1917; McCain and others 1986; Stack and Sinclair 1975; Warcup 1951a; Wright and others 1963).

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However, investigations regarding epidemiology of *Fusarium* spp. in container-grown conifer seedling production are few (Bier 1942; James and others 1987; James and others 1989; Landis 1976; McCain 1978; Pawuk and Barnett 1974). In northern Rocky Mountain nurseries, *Fusarium* spp. cause important container seedling diseases (James 1986). Diseases include seed decay (James 1986; James 1987b), damping-off of young germinants (James and others 1988; Landis 1976; Pawuk and Barnett 1974), and root diseases of older seedlings (James and Gilligan 1985; James and others 1988; Pawuk and Barnett 1974). *Fusarium* spp. can often be found on roots of containerized conifer seedlings throughout much of the nursery growth cycle even though infected seedlings may not display disease symptoms (James and Gilligan 1988; James and others 1988; James and others 1987). It is unclear how important *Fusarium* root infection may be in the survival of outplanted seedlings. Some work indicates these fungi are eliminated from roots of bareroot pine seedlings shortly after planting (Smith 1967). Conversely, our experience indicates that *Fusarium* spp. may persist on plug roots of container seedlings for at least two growing seasons following outplanting (James and others, unpublished), although these fungi have probably not been responsible for any seedling mortality during the evaluation period. However, recent experience (James 1987a; James 1989) indicates that container seedlings transplanted to agricultural or nursery soil may be killed by *Fusarium* carried on their roots.

Although most conifer species are susceptible to damage by *Fusarium* spp., Douglas-fir (*Pseudotsuga menziesii* (Mirb.)Franco) is often most severely damaged (Bloomberg 1971; Bloomberg 1973; James and others 1987; James and others 1988; Landis 1976; Lock 1973; Salisbury 1954; Shea and Rediske 1961; Van den Driessche 1963). Most northern Rocky Mountains nurseries encounter some losses from *Fusarium* during each crop. Losses are persistent and seem related to cleanliness of seed sown (James 1986; James 1987b), age and cleanliness of containers used (James, Dumroese and Wenny 1988; James, Gilligan and Reedy 1988), sanitary precautions taken within greenhouses (James 1986; McCain 1978), and the extent and type of fungicides applied (James 1988; Pawuk and Barnett 1974).

Attempts to control these diseases are often unsuccessful because of unfamiliarity with important aspects of disease epidemiology in container conifer seedling production. Therefore, investigations were undertaken to elucidate basic disease characteristics incited by *Fusarium* spp. on container-grown Douglas-fir. Characteristics such as inoculum sources, infection, and symptom production were investigated earlier (James and others 1987). Koch's postulates were completed for selected *Fusarium* isolates on Douglas-fir seedlings and reported (James and others 1989). This report describes the abundance and characteristics of various fusaria isolated from Douglas-fir seed and seedlings.

MATERIALS AND METHODS

Detailed procedures for isolating the various fusaria were previously described (James and others 1987). Isolations were made from seed and seedlings from the USDA Forest Service Nursery, Coeur d'Alene, Idaho and the University of Idaho Research Nursery, Moscow. Isolations were made from surface sterilized plant roots (10 percent aqueous bleach; active ingredient = 0.0525 percent sodium hypochlorite) onto a selective agar medium for *Fusarium* (Komada 1975). Roots were washed several minutes under running tap water to remove adhering growing medium particles prior to surface sterilization. Plates were usually incubated at about 22-24°C for 7-10 days under 12-hour diurnal cycles of cool, fluorescent light. Emerging fungi were transferred to potato dextrose agar (PDA) slants for storage until identified. Single-spore transfers were performed for these isolates after several months of refrigerated storage.

Procedures outlined by Nelson and others (1983) were used to identify isolates. Single spore isolates were prepared from macro or microconidia germinating on 2 percent water agar (WA). Spore solutions were prepared by flooding PDA plates with sterile distilled water, serially diluting suspensions and pipetting 1-2 ml of the suspension onto WA. After several hours, germinating conidia were transferred to PDA slants or plates and

incubated as described above. After PDA cultures were 7-10 days old, mass transfers of mycelium were made onto carnation leaf agar (CLA) which facilitated uniform production of macroconidia within sporodochia (Fisher and others 1982). From CLA plates, size, shape, and type of conidia produced by each isolate could be determined. Also, coniophore morphology, production of aerial mycelium and chlamydospores were determined for each isolate. Forty isolates were also characterized by evaluating their ability to grow at different temperatures (Salisbury 1952; Timmer 1982; Tint 1945c). *Fusarium* isolates, representing four species (*F. oxysporum, F. acuminatum, F. avenaceum, F. sambucinum*), were selected from those obtained from Douglas-fir seed and seedlings and grown on PDA within growth chambers at four temperatures (5, 15, 27, and 34°C). Inoculum consisted of 5 mm diameter circular plugs of mycelium cut from the advancing margin of 7 day-old cultures. Inoculum plugs were placed in the center of 90 mm diameter test plates, each containing 20 ml of PDA. Test plates were incubated for 10 days at the specific temperature (+ or - 2°C) under 12 hr. diurnal cycles of cool, fluorescent light. Each isolate at each temperature was replicated on five plates. After incubation, diameter of linear growth over the agar surface was measured (two measurements per plate were averaged). Average growth for the five replicates was calculated.

RESULTS

In order of abundance, the five *Fusarium* species isolated from Douglas-fir seed and seedlings were *F. acuminatum* Ell. & Ev., *F. oxysporum*, *F. avenaceum* (Fr.) Sacc., *F. sambucinum* Fuckel, and *F. tricinctum* (Corda) Sacc. Of 459 isolates examined, almost 90 percent were either *F. acuminatum* or *F. oxysporum* (table 1). These two species were isolated from seed or seedlings from each seedlot at both nurseries as well as from roots of greenhouse weeds. *Fusarium avenaceum* and *F. sambucinum* were isolated from seed and seedlings representing most seedlots; however, *F. tricinctum* was obtained from seedlings from only one seedlot at each nursery.

Abundance of each *Fusarium* species on seed, diseased germinants, root diseased and non-diseased seedlings and on roots of greenhouse weeds is summarized in table 2. *Fusarium oxysporum* was most commonly isolated from the roots of diseased seedlings (usually a few months old) and roots of non-diseased seedlings and greenhouse weeds. This species was isolated at lower levels from seed and diseased germinants, although some isolates were quite capable of causing seed decay and damping-off in pathogenicity tests (James et al. 1989). On the other hand, *F. acuminatum* and *F. avenaceum* were most commonly isolated from seed and diseased germinants and encountered less frequently on older seedlings. *Fusarium sambucinum* was isolated mostly from seed and the roots of non-diseased seedlings; the only isolates of *F. tricinctum* were obtained from roots of non-diseased seedlings.

Linear growth rates of forty *Fusarium* isolates at different temperatures are summarized in table 3 (individual rates for each isolate is given in table 5 - Appendix). None of the *F. oxysporum* isolates grew at the lowest temperature (5°C), but most grew significantly faster at the highest temperature (34°C) than the other three species tested. Several isolates of *F. acuminatum* grew faster at the "standard" temperature (27°C) than other species, although a few *F. oxysporum* isolates grew nearly as fast (table 5). Growth of the *F. avenaceum* and *F. sambucinum* isolates were slower at the "standard" temperature than most isolates of either *F. oxysporum* or *F. acuminatum*. However, isolates of *F. sambucinum* displayed the greatest ability to grow at both high and low temperature extremes.

DISCUSSION

Many previous investigations of *Fusarium* diseases on bareroot conifer seedlings in nurseries (Bloomberg 1971; Hamm et al. 1987; Hocking 1968: Holtzmann 1955; Kozlowska 1962; Rathbun 1918; Salisbury 1954; Vaartaja 1967; Vaartaja and Cram 1956) conclude *F. oxysporum* is the major species associated with diseased seedlings. However, our evaluations of container-grown Douglas-fir seedlings at two nurseries in northern Idaho indicate

at least one other *Fusarium* species was at least equally important. In fact, *F. acuminatum* was isolated more frequently than *F. oxysporum* from healthy and diseased seed and young diseased germinants.

Fusarium oxysporum was most commonly isolated from roots of seedlings which were several months old; this species colonized roots of diseased as well as non-diseased (without symptoms) seedlings. Isolates of *F. oxysporum* are common colonizers of epidermal and cortical cells of a variety of plants, including conifer seedlings (Bloomberg 1966; Gerik and Huisman 1985; Haware and Nena 1982; James and others 1986; Park 1959; Schneider 1984; Timmer 1982). They are also important rhizosphere residents (Park 1959; Timmer 1982). Although soilborne, strains of *F. oxysporum* are not strong competitors with many other soil microorganisms and are best adapted as rapid colonizers of plant roots (Gordon 1959; Park 1963; Toussoun 1975; Toussoun and others 1969; Warcup 1951b). While infecting plant roots, *F. oxysporum* may elicit host disease symptoms. However, some isolates quite often may not cause noticeable detrimental effects of hosts (Bloomberg 1966; Hansen and Hamm 1988; James and Gilligan 1988; Schneider 1984; Wellman and Blaisdell 1941). Development of disease syumptoms in bareroot seedlings may be related to levels of host susceptibility, likely mediated by environmental conditions such as temperature, soil pH, and moisture (Bloomberg 1981; Bloomberg 1985; Brownell and Schneider 1985; Salisbury 1952; Timmer 1982; Tint 1945b; Tint 1945c). Other important factors affecting production of disease symptoms include genetic determinants of host susceptibility (Bloomberg 1985; Tint 1945b) and pathogen aggressiveness (Bloomberg 1976; Bloomberg and Lock 1972; Tint 1945a).

Fusarium oxysporum is characterized on some hosts as specific strains (formae specialis), capable of colonizing and causing disease of specific cultivars (Armstrong and Armstrong 1975; Bloomberg and Lock 1972; Hanioja 1969; Matuo and Chiba 1966; Messiaen and Cassini 1981; Nelson and others 1983). It is possible such specialization may exist for isolates attacking different species of conifer seedlings, although the specifics of such associations are unclear. Isolates causing disease of conifer seedlings have previously been designated as formae specialis *pini*, regardless of host species attacked or type of disease caused (Booth 1971; Lock 1973; Snyder and Hansen 1940). Matuo and Chiba (1966) presented evidence to designate different formae specialis for *F. oxysporum* isolates consistently attacking different conifer species. Our experience is that *F. oxysporum* isolated from conifer seedlings comprises different morphological types in culture as well as variability in pathogenicity (James and others 1989).

Of the *F. oxysporum* isolates from Douglas-fir seed and seedlings examined in this study, three general morphological types were characterized in culture. All three types contained certain characteristics conforming to general descriptions of *F. oxysporum* in taxonomic treatises (see Appendix). For example, all three culture types produced abundant oval to ellipsoid microconidia borne in false heads at the ends of short, mostly unbranched monophialides. Abundant chlamydospores were produced by most isolates after growing in culture several weeks. Macroconidia were not always abundantly formed on PDA; however, most isolates formed uniform macrocondia within orange sporodochia on CLA. Probably the most common cultural type (type I) produced abundant white, floccose aerial mycelium over the entire culture surface. Level of violet pigmentation varied among different isolates of this culture type, i.e., some produced very dark pigment, whereas others produced little or no pigment beneath colonies. Several variants of type I cultures produced a more appressed aerial mycelium interspersed with abundant violet-black sclerotia. In these variants, intense violet pigmentation beneath the colony was the rule. Type II cultures of *F. oxysporum* produced a peach-colored to orange colony on PDA. These usually had little aerial mycelium and abundant sporodochial production (pionnotal) and rarely produced the violet pigmentation characteristic of type I cultures. Type III cultures produced deep violet pigment often extending well into the abundant aerial mycelium. The aerial mycelium of type III was much finer (feathery) than the coarse and powdery appearance of type I cultures. Sclerotia were usually not common in type III and sporodochia and chlamydospores were also rare. In some isolates of this third type, aerial mycelium became appressed after several weeks in culture; some resembled cultures of *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas comb. nov. (Kuhlman and others 1978).

Examples of each culture type were evaluated in temperature-growth tests described in this report and pathogenicity tests described previously (James and others 1989). Consistent differentiation of the three types based on growth at different temperatures and pathogenicity to Douglas-fir germinants and seedlings was not evident.

Characterization of *F. oxysporum* isolates from conifer seedlings, using technology developed for agricultural isolates, would be very useful. Such tests should include vegetative compatibility analyses using heterokaryon formation of minimal media mutants (Puhalla 1985) and isozyme polymorphism characterization (Bosland and Williams 1987; Glynn and Reid 1969; Meyer and Renard 1969). These tests may help elucidate relative genetic relatedness of different isolates so pathogenic potential of different strains could be determined (Bosland and Williams 1987; Correll and others 1986). It is possible genetic differences among *F. oxysporum* isolates are major determinants of pathogenic behavior on conifer seedlings (Puhalla 1981). It is also possible most strains are capable of causing disease whenever host susceptibility and environmental conditions are conducive (Bloomberg 1985).

Although *F. acuminatum* has previously been associated with conifer seedling diseases (Hildebrand 1985; James 1986; Rathbun-Gravatt 1925), it is usually considered a minor pathogen. Our investigations of container-grown Douglas-fir seedlings isolated this species frequently and found it capable of aggressively attacking seedlings (James and others 1989). It is possible other investigators, when evaluating its role as a pathogen of conifer seedlings, labeled this species "*F. roseum*" due to its deep carmine pigmentation (Buxton, Sinha and Ward 1962; Hamm and Hansen 1986; Hansen and Hamm 1988).

Most *F. acuminatum* isolates obtained were fairly uniform in colony morphology (see Appendix for species description), fast growing (although not at high temperatures like *F. oxysporum*), and produced abundant aerial mycelium that was initally white, but became tinted with carmine pigmentation as the culture aged. Often an ochraceous color was produced in the aerial mycelium after a few weeks. Some isolates produced orange sporodochia on PDA, but many did not. On CLA, all isolates produced thin, strongly curved, sickle-shaped macroconidia, with an elongated apical cell. Chlamydospores were common in most isolates, but were not formed until after a few weeks in several isolates. None of the examined isolates produced microconidia.

Another closely related species frequently isolated was *F. avenaceum*. This species has also been previously associated with conifer seedling diseases (Bier 1942; Crandall 1938; Crandall and others 1945; Rathbun-Gravatt 1925; Tint 1945a). It has been an especially important pathogen of agricultural crops (Bretag 1985; Burgess and others 1975; Duthie and others 1986). Morphologically, this species closely resembles *F. acuminatum* (see Appendix). The major criterion separating the two species is production of chlamydospores by *F. acuminatum* and the lack of these spores by *F. avenaceum* (Nelson and others 1983). Unfortunately, some strains of *F. acuminatum* are very slow in producing chlamydospores in culture and these may be mistakingly identified as *F. avenaceum* (Nelson and others 1983). However, the relative consistency of slower growth of *F. avenaceum* isolates at most temperatures (table 5) may be another factor separating these two species.

Fusarium sambucinum has rarely been reported as a pathogen of conifer seedlings (Bier 1942; Pomerleau 1934; Tint 1945a). However, this species is an important agricultural pest and is particularly important as a producer of powerful toxins (Bennett 1935; Griffin and Pass 1969; Schneider and Seaman 1974). Isolates tested on Douglas-fir seed and seedlings (James and others 1989) were not very pathogenic. This species was differentiated from other carmine-pigment producing *Fusarium* species by macroconidial morphology (produced within orange sporodochia on CLA) and the lack of microconidia (see Appendix). Macroconidia were thick, slightly-curved and without an apical cell extension. Most isolates produced abundant chlamydospores in culture after a few weeks.

Fusarium tricinctum was rarely encountered in this study and only isolated from roots of non-diseased seedlings. Although this species has previously been associated with conifer diseases (Bennett 1935; Kuhlman and others 1978), it is more frequently an inhabitant of agricultural soils (Kommedahl and others 1975; Miller and others 1957) and a weak pathogen of cereals and other agricultural crops (Booth 1971; Palmer and Kommedahl 1969). *Fusarium tricinctum* was differentiated from other carmine pigment-producing species by its abundant production of two microconidial types (lemon to pear-shaped and spindle-shaped) borne on monophialides (see Appendix).

Temperature-growth relationships were used to ascertain preferences of certain *Fusarium* species for activity at different temperatures. Our results confirmed previous studies (Harling and others 1988; Kozlowska 1962; Tint 1945c) which indicate that *F. oxysporum* isolates are generally "warm weather fungi" which grow faster at higher temperatures. Studies of disease resistance to *F. oxysporum* (Harling and others 1988) indicated expression of disease symptoms was related to temperature, i.e., at higher temperatures, host resistance broke down and disease symptoms developed rapidly. Similar behavior may occur in container-grown conifer seedlings where temperature may be an important factor affecting disease symptom expression.

A few individual isolates of *F. acuminatum* and *F. avenaceum* were capable of growing at either high or low temperature extremes. However, most grew between 15-27°C. Conversely, all *F. sambucinum* isolates grew at all temperatures tested, indicating a wide range of species adaptability to temperature extremes.

Evaluating environmental effects on pathogenic potential of these *Fusarium* species would be valuable in enhancing our understanding of how these fungi function during typical growth cycles of container-grown seedlings. Such factors as temperature, moisture stress, pH, and nutritional effects should be investigated as well as characterization of individual fungal species based on vegetative compatibility, isozyme analyses, and host range potential. This information will greatly improve our understanding of the epidemiology of these important conifer seedling pathogens.

ACKNOWLEDGEMENTS

The authors wish to thank W. H. Livingston for *Fusarium moniliforme* isolates used for comparisons with those obtained from Douglas-fir seed and seedlings. We also thank P. B. Hamm for critical review of the manuscript.

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Problems of *Fusarium* Taxonomy

Throughout this evaluation, the taxonomic guide of Nelson, Toussoun and Marasas (1983) was used along with occasional reference to species descriptions of Gerlach and Nirenberg (1982) and Domsch and others (1980). The taxonomic scheme of Nelson, Toussoun and Marasas provides a workable system applicable when investigating various fusaria, if certain precautions are taken and if guidelines outlined in their manual are closely followed (such as growth on CLA and PDA and transferring single-spore cultures). Other taxonomic treatises exist for this important group of plant pathogenic fungi (Bilai 1955; Booth 1971; Gordon 1959, 1960; Joffe 1974; Messiaen and Cassini 1981; Snyder and Hansen 1940; Wollenweber 1931; Wollenweber and Reinking 1935). However, most have certain disadvantages when trying to characterize fusaria (Booth 1984). The current system of Nelson, Toussoun and Marasas is mostly a composite of many of these other systems, assimilated into a workable guide useful to most workers.

Since taxonomy is basically a man-made system of nomenclature formulated to establish distinct boundaries between organisms actually existing in nature as an evolutionary continuum (Mayr 1982), any system devised is likely to exhibit some artificiality. However, if characteristics that are repeatable and predictable over time are selected, and if these characteristics designate a specific taxon, then such a system can be used to satisfactorily describe species. For this evaluation, the characteristics outlined in table 4 were consistently used to classify the *Fusarium* spp. isolated from Douglas-fir seed and seedlings. A detailed taxonomic description of each *Fusarium* spp. isolated during this evaluation follows.

Fusarium acuminatum Ell. & Ev. (Section *Gibbosum* Wollenw.)

Selected synonymy

Fusarium equiseti (Corda)Sacc. var. *acuminatum* (Ell. & Ev.)Bilai
Fusarium gibbosum Appel & Wollenw. emend. Bilai var. *acuminatum* (Ell. & Kellerm.)Bilai
Fusarium roseum Lk emend. Snyd. & Hans. "*Acuminatum*" S. & H.
Fusarium roseum Lk emend. Snyd. & Hans. var. *gibbosum* (Wollenw.) Messianen & Cassini

Fusarium scirpi Lamb. & Fautr. var. *acuminatum* (Ell. & Ev.) Wollenw.

Teleomorph: *Gibberella acuminata* (Wollenw.)

Colony morphology: On PDA growth is very rapid (7.8-8.2 cm diam in 6 days at 25°C. Aerial mycelium usually dense, whitish, pink, pale ochraceous, and occasionally partly carmine tinged with stroma pigments.

Pigmentation: Variable among different strains, but mostly deep carmine to blood red; some strains may have ochraceous, amber to brown pigmentation.

Sclerotia: Occasionally present as buff-brown to dark brown-blue bodies of variable shape and size.

Conidia: Microconidia are lacking in most strains and sparse in others. Macrocondia are thin, strongly falcate, usually with the widest point about one-third of the distance from the base. Macroconidial apical cells are distinctly elongate and basal cells are foot shaped.

Conidiophores: Branched and unbranched monophialides.

Chlamydospores: Common in most strains (although formed very slowly in others), predominantly intercalary in chains, globose to subglobose, and usually smooth walled.

General: The most distinguishing characteristic of *F. acuminatum* is the shape of the macroconidia and presence of chlamydospores. It is often difficult to separate *F. acuminatum* from *F. avenaceum* because the shape of macroconidia of these two species grade into one another. Therefore, the major distinction between these two species becomes presence of chlamydospores. However, chlamydospore formation may be very slow in some strains of *F. acuminatum*. Cultures of *F. acuminatum* are generally quite stable, although sector variants may occur.

Fusarium avenaceum (Fr.)Sacc. (Section *Roseum* Wollenw.)

Selected synonomy

Fusarium roseum Lk. emend. Snyd. & Hans. "Avenaceum" S. & H.

Fusarium roseum Lk. emend. Snyd. & Hans. var. *avenaceum* (Sacc.)Snyd. & Hans. [M. & C.]

Teleomorph: *Gibberella avenacea* Cook

Colony morphology: On PDA growth is moderately fast growing (5.0-8.0 cm diam. in 10 days at 25°C). Aerial mycelium is dense and varies in color from white, tan, rose, to reddish-brown.

Pigmentation: Pink, rose to carmine, yellowish to ochraceous; becomes red-brown to brown with age.

Sclerotia: Present in most strains, small, cauliflower-like, beige to bluish-black.

Conidia: Microconidia are usually lacking or very sparse. Macroconidia are very long, slender, thin-walled, with an apical cell that is elongated and a distinct pedicellate basal cell.

Conidiophores: Branched and unbranched monophialides.

Chlamydospores: Absent

General: The most distinguishing characteristics are the size and shape of the macroconidia produced on CLA and the lack of chlamydospores. This species may be confused with *F. acuminatum* (see discussion above). This species is represented by many strains which differ considerably in colony morphology. Some strains degenerate by subculturing. Culture variants may be pionnotal, reddish without aerial mycelium or with white aerial mycelium arranged in radiating strands. Some variants may be sterile.

Fusarium oxysporum Schlecht. (Section *Elegans* Wollenw.)

Selected synonymy

Fusarium bulbigenum Cooke & Massee

Fusarium conglutinans Wollenw.

Fusarium redolens Wollenw.

Fusarium tracheiphilum E. F. Smith

Fusarium vasinfectum Atkinson

Teleomorph: None known

Colony morphology: On PDA growth is rapid (7.5-8.0 cm diam in 8 days at 25°C, although there are considerable differences among strains. Aerial mycelium is usually abundant, loosely floccose and becoming felt-like. In some strains the mycelium is sparsely developed and appressed and in others the mycelium quickly forms expanded pionnotal slimes.

Pigmentation: Variable, extending from pale beige, salmon, rose to strong purple; sometimes vinaceous and dark bluish violet with greyish blue or greenish blue areas interspersed.

Sclerotia: Variable in occurrence, of different size, globose to subglobose, pale, buff-brown, greyish-blue or greenish blue to deep violet.

Conidia: Microconidia are abundant, generally single-celled, oval to kidney-shaped, and produced in false heads. Macroconidia are usually abundant, only slightly falcate, thin-walled, with an attenuated apical cell and a foot-shaped basal cell. Macroconidia are initially solitarily scattered throughout the aerial mycelium, but may later form in pale salmon to bright orange sporodochia or in pionnotal slimes.

Conidiophores: Microconidia borne on short, mostly unbranched monophialides. Macrocondia usually borne on short, unbranched or branched monophialides.

Chlamydospores: Generally abundant in most isolates, formed singly or in pairs, smooth or rough-walled, globose to subglobose. Some isolates produce short chains or occasional clusters of chlamydospores.

General: *Fusarium oxysporum* exhibits a variety of morphological features on PDA. It can also mutate frequently in culture. Isolates may progressively become more mycelial (an increase in aerial mycelium with a corresponding decrease in pigmentation, sclerotia or sporodochia) or may become pionnotal (the aerial mycelium becomes appressed and macroconidia are produced in pionnotes that give the colony a wet, orange or yellow appearance (Waite and Stover 1960)

Fusarium sambucinum Fuckel (Section *Discolor* Wollenw.)

Selected synonymy

Fusarium bactridioides Wollenw.
Fusarium roseum Lk. emend. Snyd. & Hans. "Sambucinum" (S. & H.)
Fusarium roseum Lk. emend. Snyd. & Hans. var. *sambucinum* (Fuckel) Snyd. & Hans. [M. & C.]
Fusarium sambucinum Fuckel var. *trichothecioides* (Wollenw.)Bilai
Fusarium sulphureum Schlecht.
Fusarium trichothecioides Wollenw.

Teleomorph: *Gibberella pulicaris* (Fr.)Sacc.

Colony morphology: On PDA growth is moderately fast growing (5.0-8.0 cm. diam. in 10 days at 25°C). Considerable differences in growth rate may occur among different strains. Aerial mycelium is usually abundant, floccose, whitish to rose or pale brown.

Pigmentation: Frequently carmine red, but may also become shades of tan to brown.

Sclerotia: Usually abundant and conspicuous in freshly-isolated cultures; generally dark brown to blue.

Conidia: Microconidia are usually absent. Macroconidia are short and stout, distinctly septate with thick walls and strongly falcate. Basal cells are usually distinctly pedicellate whereas the apical cell is constricted.

Conidiophores: Branched and unbranched monophialides.

Chlamydospores: Abundant and quickly formed in culture; may be single, in chains or clusters.

General: The most distinguishing characteristic is the shape of the macroconidia on CLA. This species comprises a morphologically large and varied group. Pionnotal and mycelial mutants occur naturally or form in culture.

Fuarium tricinctum (Corda)Sacc. (Section *Sporotrichiella* Wollenw.)

Selected synonymy

Fusarium citriforme Jamalainen

Fusarium sporotrichiella Bilai var. *tricinctum* (Corda)Bilai

Fusarium sporotrichioides Sherb. var. *tricinctum* (Corda)Raillo

Teleomorph: *Gibberella tricincta* El-Gholl, McRitchie, Schoulties & Ridings

Colony morphology: On PDA moderately fast growing (4.5-6.0 cm. diam. in 10 days at 25°C). Aerial mycelium is dense, giving the culture a cushion-like appearance, margins irregularly lobed, color white to carmine to ochraceous. Orange sporodochia are common as the colony ages.

Pigmentation: Intensely carmine to dark red.

Sclerotia: Abundant in some strains, brown to blue-grey or black.

Conidia: Two types of microconidia are formed: (1) lemon to pear-shaped (napiform to pyriform) and (2) spindle-shaped with 0-1 septa and papillate at their base. Macroconidia are slender, strongly falcate with a basal cell that is distinctly foot-shaped or notched.

Conidiophores: Branched and unbranched monophialides.

Chlamydospores: Not abundant in most strains; formed singly or in chains.

General: The most distinguishing characteristic is production of two types of microconidia borne on monophialides. It is also slower growing than several similar species.

Table 1.--Abundance of *Fusarium* species on Douglas-fir seed and seedlings representing selected seedlots from the USDA Forest Service Nursery and the University of Idaho Research Nursery.

	Percent of *Fusarium* isolates					
Seedlot	Number isolates	*oxysporum*	*acuminatum*	*avenaceum*	*sambucinum*	*tricinctum*
USDA Forest Service						
2682	46	34.8	47.8	15.2	0	2.2
2741	87	24.1	59.9	14.9	1.1	0
4010	41	56.1	34.1	4.9	4.9	0
4486	82	37.9	52.4	7.3	2.4	0
6070	87	35.6	50.7	8.0	5.7	0
University of Idaho						
DAW	32	65.6	28.1	0	6.3	0
FN	31	67.8	25.8	3.2	3.2	0
IDL	34	26.6	50.0	2.9	17.6	2.9
Weeds[1]	19	52.6	47.4	0	0	0
Total isolates	459	183	218	37	19	2
Percent of isolates	100.0	34.9	47.5	8.1	4.1	0.4

[1]Weeds on the floor within greenhouses at the USDA Forest Service Nursery, Coeur d'Alene.

Table 2.--**Abundance of *Fusarium* species on Douglas-fir seed, diseased and nondiseased seedlings and greenhouse weeds from the USDA Forest Service Nursery and the University of Idaho Research Nursery.**

	Percent of *Fusarium* isolates					
Isolation source	Number *isolates*	*oxysporum*	*acuminatum*	*avenaceum*	*sambucinum*	*tricinctum*
Seed						
Healthy	69	23.2	59.4	8.7	8.7	0
Decayed	51	27.4	60.9	7.8	3.9	0
Diseased germinants						
Damped-off	79	16.5	65.8	17.7	0	0
Cotyledon blight	57	24.6	61.4	12.3	1.7	0
Root-diseased Seedlings	62	77.5	17.7	1.6	3.2	0
Non-diseased Seedlings	122	55.7	32.0	4.1	6.6	1.6
Greenhouse Weeds	19	52.6	47.4	0	0	0

Table 3.--Linear growth of selected *Fusarium* isolates from Douglas-fir seed and seedlings at different temperatures on potato dextrose agar.

		10-Day linear growth (mm)							
Fusarium species	Number isolates	5°C Average	5°C Range	15°C Average	15°C Range	27°C Average	27°C Range	34°C Average	34°C Range
oxysporum	20	O A[1]	0-0	41.9 AB	33.8-55.0	91.6 B	80.0-114.3	20.9B	0.45.8
acuminatum	7	2.5 AB	0-17.4	58.8 B	43.0-75.2	102.0 B	90.8-121.4	3.3 A	0-9.4
avenaceum	9	3.6 AB	0-9.7	44.5 AB	15.9-70.9	63.3 A	50.0-77.9	0.4 A	0-3.7
sambucinum	4	5.3 B	4.0-5.6	23.6 A	17.2-28.3	61.1 A	51.8-78.9	6.9 A	4.0-9.0
All species	40	1.8	0-17.8	43.6	15.9-75.2	84.2	50.0-121.4	11.8	0-45.8

[1]Within each column, means followed by the same capital letter are not significantly different (P=0.05) using Duncan's Multiple-Range Comparison Test.

Table 4.--Major characteristics used to place *Fusarium* isolates from Douglas-fir seed and containerized seedlings within designated taxa.[1]

	Fusarium species				
Characteristic	*acuminatum*	*avenaceum*	*oxysporum*	*sambucinum*	*tricinctum*
Growth rate	Rapid[2]	Rapid	Rapid	Rapid	Rapid
Color aerial mycelium	White Pink	White Pink, Tan	White Orange, Violet	White Pink, Tan	White
Pigment under colony	Carmine Yellow	Carmine Yellow	Violet Orange, None	Carmine	Carmine
Sporodochial color	Red-brown Orange	Red-brown Orange	Orange Tan, Cream	Orange	Orange
Macroconidia shape	Thin, Very Falcate	Thin, Very Falcate	Thin, Short	Stout, Thick Walls	Stout, Slight Falcate
Microconidia present	No	No	Yes	Yes	Yes
Chlamydospores present	Yes	No	Yes	Yes	Yes

[1]Growth rate, color of aerial mycelium, and colony pigmentation determined on PDA. All other characteristics determined on CLA.

[2]Greater than 70 mm diameter after 10-14 days at 22°.

Table 5.--Linear growth of specific *Fusarium* isolates from Douglas-fir seed and seedlings at different temperatures on potato dextrose agar.

			10-Day linear growth (mm)			
Species	Isolate	Source[1]	5°	15°C	27°C	34°C
oxysporum	16 C	CDA-DF	0	41.0	100.0	11.2
	23 C	CDA-DOR	0	37.7	80.0	39.3
	23 G	CDA-DS	0	53.6	92.0	0
	27 B	CDA-NDR	0	39.7	92.7	9.8
	29 G	UI-NDS	0	44.2	98.1	6.9
	42 C	UI-NDR	0	55.0	86.7	22.1
	43 B	CDA-NDR	0	40.9	87.5	16.4
	49 H	CDA-NDR	0	48.0	114.3	12.6
	49 K	CDA-DR	0	35.0	96.6	18.5
	49 S	CDA-DR	0	40.5	88.0	16.5
	56 M	UI-NDR	0	33.8	81.5	42.5
	62 B	CDA-NDR	0	45.3	87.5	21.8
	62 D	CDA-NDR	0	45.7	88.1	13.4
	69 F	UI-NDR	0	40.6	86.6	18.0
	70 A	UI-DR	0	36.6	81.9	20.6
	70 B	UI-DR	0	38.1	84.8	45.8
	74 B	UI-NDR	0	41.0	102.8	31.2
	74 D	UI-NDR	0	35.2	93.5	27.6
	75 B	CDA-DR	0	46.8	98.3	21.6
	75 C	CDA-DR	0	38.7	91.6	18.9
	Average	--	0	41.9	91.6	20.9
acuminatum	14 B	CDA-DOR	0	43.0	96.1	0
	18 M	CDA-DOR	0	65.3	90.8	0
	26 G	CDA-DR	0	48.1	102.4	0
	27 D	CDA-NDR	0	62.0	95.7	8.0
	28 K	CDA-NDR	0	64.3	114.4	0
	50 A	CDA-NDR	17.4	75.2	121.4	9.4
	69 L	UI-NDR	0	53.5	100.0	5.8
	Average	--	2.5	58.8	103.0	3.3
avenaceum	5 A	CDA-NDS	9.7	15.9	54.4	0
	7 H	CDA-NDS	0	70.9	75.3	0
	13 I	CDA-DOR	0	55.9	65.7	0
	18 I	CDA-DOR	0	52.7	57.8	0
	19 J	CDA-DOR	7.5	48.1	52.9	0
	19 N	CDA-DOR	0	40.7	59.0	0
	20 C	CDA-NDR	0	32.3	76.7	0
	26 C	CDA-DS	6.1	24.6	50.0	3.7
	46 E	CDA-NDR	9.2	59.4	77.8	0
	Average	--	3.6	44.5	63.3	0.4
sambucinum	19 I	CDA-DS	5.5	28.3	51.8	9.0
	30 E	UI-NDS	5.0	23.3	54.8	8.8
	37 A	CDA-DOR	5.3	17.2	78.9	5.7
	43 E	CDA-NDR	5.6	25.7	59.1	4.0
	Average	--	5.3	23.6	61.1	6.9

[1]**CDA** = *USDA Forest Service Nursery, Coeur d'Alene, Idaho;* **UI** = *University of Idaho Research Nursery, Moscow, Idaho;* **DF** = *Roots of greenhouse weeds;* **DOR** = *Damped-off seedling;* **DS** = *Decayed seed;* **NDR** = *Root of nondiseased seedling;* **NDS** = *Nondiseased seed;* **DR** = *roots of diseased seedling*